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(54) Title: METHOD OF TREATING VIRAL INFECTIONS USING LFA-1

(57) Abstract

The invention features substantially pure recombinant β -subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, an analog thereof, or a fragment thereof composed of at least 10 % of a contiguous sequence of the β -subunit; a cDNA sequence encoding therefor; and a vector containing a DNA sequence encoding therefor. The invention also features any monoclonal antibody raised against the recombinant β -subunit of human LFA-1 and a method of treating rhinoviral infections using LFA-1.

p150.95 β Subunit

P-61 sequence	L Y E N N I Q P I F A V T S
Deduced sequence	K L A E N N I Q P I F A V T S
P-20 sequence	(T/C) D T G Y I G K
Deduced sequence	R C D T G Y I G K
P-18 sequence	S S Q E L E G S (T/C) (R)
Deduced sequence	R S S Q E L E G S C R

Mac-1 β Subunit

M-58 sequence	L L V F A T D D G F H F
Deduced sequence	R L L V F A T D D G F H F
M-52 sequence	X A V G E L S E X(S) X N
Deduced sequence	K S A V G E L S E D S S N

LFA-1 β Subunit

L56a sequence	E C Q P P F A F R
Deduced sequence	K E C Q P P F A F R
L56b sequence	L I Y G Q Y C E(C) D T I
Deduced sequence	K L I Y G Q Y C E C D T I
L-65 sequence	V F L D H N A L P
Deduced sequence	R V F L D H N A L P

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TITLE OF THE INVENTION:

"Method of Treating Viral Infections Using LFA-1"

This application is a continuation-in-part of U.S. Patent Application Serial No. 07/019,440; filed on February 26, 1987.

Background of the Invention

The work described herein was performed with the aid of government funding, and the government therefore has certain rights in the invention. Specifically, the work was supported by N.I.H. grants 31798 and AI 05877.

This invention relates to cellular adhesion.

Cellular adhesion is a critical function for guiding migration and localization of cells, and for maintaining the integrity of the body. Receptors for extracellular matrix components such as fibronectin, laminin, and vitronectin mediate cellular adhesion during morphogenesis and wound healing. In the immune system, regulatory networks require intimate cell-cell interaction among lymphocytes and antigen-presenting accessory cells, and cell-mediated cytolysis involves direct contact between the effector cell and virally-infected or transformed target cells. Leukocyte-endothelial interactions are important in leukocyte mobilization into inflammatory sites and in lymphocyte recirculation. These cellular adhesion reactions are mediated

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in part by a family of structurally related glycoproteins, LFA-1, Mac-1, and p150,95, all of which share a common β -subunit (hereinafter referred to as the β -subunit of human LFA-1). Springer et al., 314 Nature 540, 1985; Springer et al., "The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system" Ann. Rev. Immunol. Vol. 5, 1987; both hereby incorporated by reference.

Summary of the Invention

In general, the invention features a) substantially pure recombinant β -subunit of a human glycoprotein concerned with cellular adhesion, or b) a biologically active fraction of this β -subunit, c) an analog of the β -subunit, or c) a fragment of the β -subunit, composed of at least 10% of a contiguous sequence of the β -subunit. The invention also features a cDNA sequence encoding for the β -subunit; and a vector containing a DNA sequence encoding therefor. By recombinant subunit is meant the polypeptide product of recombinant DNA encoding the β -subunit, i.e., the polypeptide expressed from DNA which is not in its naturally occurring location within a chromosome. By natural subunit is meant that subunit produced naturally *in vivo* from naturally occurring and located DNA. By analog is meant a polypeptide differing from the normal polypeptide by one or more amino acids, but having substantially the biological activity of the normal polypeptide. The invention also features any monoclonal antibody (MAb) raised against the recombinant β -subunit, a biologically active fraction, an analog, or a fragment thereof composed of at least 10%, preferably at least 80%, of a contiguous sequence of the β -subunit of a human glycoprotein.

The cDNA sequence encoding the LFA-1 β -subunit or a fragment thereof may be derived from any of the naturally

occurring genes encoding it, or synthesized chemically. Variations in this sequence which do not alter the amino acid sequence of the resulting protein, or which do not significantly alter the biological activity of the protein, are also acceptable, and are within this invention.

Preferably the human glycoprotein is LFA-1, Mac-I or p150.95.

As will be described in more detail below, the invention permits the diagnosis and treatment of a variety of human disease states.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

Description of the Preferred Embodiments

The drawings are first briefly described.

Drawings

Fig. I is the DNA coding sequence of the alpha-subunit of LFA-1, Mac-I and p150.95. Potential N-glycosylation sites are marked with triangles.

Figure 2 is a comparison of the amino acid sequence predicted from the cDNA in Fig. 1, and the amino acid sequence derived from enzyme digests of the alpha-subunit of LFA-1. Ambiguous determinations of amino acids are bracketed. The code for amino acids is as follows:

Ala, A	-alanine
Arg, R	-arginine
Asn, N	-asparagine
Asp, D	-aspartic acid
Cys, C	-cysteine
Gln, Q	-glutamine
Glu, E	-glutamic acid
Gly, G	-glycine
His, H	-histidine
Ile, I	-isoleucine
Leu, L	-leucine

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Lys, K	-lysine
Met, M	-methionine (start)
Phe, F	-phenylalanine
Pro, P	-proline
Ser, S	-serine
Thr, T	-threonine
Trp, W	-tryptophan
Tyr, Y	-tyrosine
Val, V	-valine

Methods

In general, the β -subunit of any of the above described related glycoproteins is isolated by standard procedures and the amino acid sequence of at least a part of it determined. From this analysis a synthetic oligonucleotide probe, corresponding to the amino acid sequence, is synthesized and used as a probe for a genomic or cDNA library containing a DNA sequence encoding the β -subunit. An example of this procedure is given below. One skilled in the art will realize that this represents only one of many methods which can be used to achieve cloning of the gene encoding the LFA-1 β -subunit.

Purification of the β -Subunit

MAb's directed against the alpha subunits of p150,95, Mac-1, and LFA-1, were used to affinity purify their respective proteins from three different sources. The p150.95 protein was purified from hairy cell leukemia spleens (Miller et al., 1986, 137 J. Immunol. 2891, hereby incorporated by reference); Mac-1 was purified from pooled human leukocytes (Miller et al., *supra*); and LFA-1 was purified from the SKW3 T cell line using TS1/22 monoclonal antibody (Sanchez-Madrid et al. 1983, J. Exp. Med. 158:586, hereby incorporated by reference).

Preparative SDS-PAGE gels were run using the method of Laemmli (Hunkapiller et al., 1983, Meth. Enzym. 91:227). 0.1 mM Na Thioglycolate was added to the upper chamber to reduce

the level of free radicals in the gel. Bands were visualized by soaking the gel for several minutes in 1 M KCl and then excised. The β -subunit was electroeluted using the apparatus and method described by Hunkapillar et al., supra. The purified protein was reduced with 2 mM DTT in the presence of 2% SDS and alkylated with 5 mM iodoacetic acid in the dark. (In some cases, the protein was reduced and alkylated prior to running the preparative gel.)

Amino acid sequencing

The above samples were precipitated using four volumes of ethanol at -20°C for 16 hr, and the protein pellet redissolved in 30-50 μ l of 0.1 M NH_4CO_3 containing 0.1 mM CaCl_2 and 0.1% zwittergent 3-14 (Calbiochem, San Diego, CA). The sample was then digested with 1% w/w trypsin for 6 hr at 37°C. At 2 and 4 hr during the incubation, additional trypsin (1% w/w) was added.

The tryptic peptides were resolved by reverse phase HPLC (Beckman Instruments) with a 0.4 X 15 cm C4 column (Vydac, Hesperig, CA), and eluted from a 2 hr linear gradient from 0 to 60% acetonitrile. 0.1% TFA was included in both the aqueous and organic solvents. The peaks were monitored at 214 and 280 nm and collected into 1.5 ml polypropylene tubes. The fractions were concentrated to 30 μ l or less on a speed-vac apparatus, and selected peptides subjected to sequence analysis using a gas phase microsequenator (Applied Biosystems, Foster City, CA).

Example: β -subunit of p150,95

p150,95 was affinity purified from the spleens of human patients with hairy cell leukemia using a monoclonal antibody specific for the alpha subunit (MW approx. 150,000, Miller et al., supra). Analysis of the purified protein by SDS-PAGE and silver staining revealed the characteristic alpha and beta

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subunit, with no significant amounts of contaminating proteins. The β -subunit band was excised from a preparative SDS-PAGE gel and electroeluted, as described above.

The N-terminus of the beta subunit was blocked and therefore could not be sequenced. Internal amino acid sequence information was obtained by digesting the β -subunit with trypsin. The tryptic peptides were resolved by reverse phase HPLC and eluted on a 60% acetonitrile gradient. Peaks analyzed by absorbance at 214 and 280 nm were collected and applied to a gas phase microsequenator.

The peptide sequences of two of these fragments is: P-61 Peptide Sequence: LeuTyrGluAsnAsnIleGlnProIlePheAlaValThrSer P-20 Peptide Sequence: ThrAspThrGlyTyrIleGlyLys.

Two strategies were adopted for constructing oligonucleotide probes. A unique sequence 39mer was designed from peptide P-61 based on human codon usage frequency (Lathe, 1985. J. Mol. Biol. 183:1). Its sequence is: 3'-GACATACTCTTGTAGGTAGGGTAGAAACGACACTGG -5'. In addition, two sets of mixed sequence probes were constructed such that every possible sequence was represented. A 20mer of 96-fold redundancy was derived from peptide P-61, and a 17mer of 192-fold redundancy was constructed based on the sequence from a different peptide fragment of the β -subunit, P-20. These sequences are given below.

20mer, Mixed Sequence 3'- ATACTATTATTATAAGTCCC -5'
G C G G C T
G

17mer, Mixed Sequence 3'- CTATGACCAATATAACC -5'
G C C G G
G G T
T T

The 39mer and the mixed sequence 20mer were used to probe a Northern blot of poly A+selected RNA from PMA-activated U937 cells. The U937 cells, J4 lymphoblastoid cells, HeLa cells,

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and CO3 cells (Springer et al., 1984, J. Exp. Med. 160:1901, an EBV-transformed cell line from a healthy donor) were grown in RPMI 1640 containing 10-15% fetal calf serum in a humidified atmosphere of 5% CO₂ and 37°C. The U937 cells were activated with 2 ng/ml PMA for three days prior to harvesting. The cells were lysed in a 4M guanidinium isothiocyanate solution, and RNA isolated in a 5.7M CsCl gradient. Poly A+ mRNA was selected with oligo (dT)-cellulose columns (Maniatis et al., Molecular Cloning: A laboratory manual, Cold Spring Harbor Laboratory, N.Y., 1982) or oligo (dT)-affinity paper (Amersham). This RNA was denatured and sized on a 1% agarose gel containing formaldehyde (Maniatis et al, supra) and transferred to nylon membranes (BioRad) in 20X SSC. A lane containing 28S and 18S ribosomal RNA from human cells or 23S and 16S rDNA from Escherichia coli was run to provide molecular weight standards.

The filters were hybridized with nick-translated probe DNA at 42°C for 18 hr in 5 x SSPE, 50% formamide, 10% dextran sulfate, 1 X Denhardt's, 0.5% SDS and 100 ug/ml denatured salmon sperm DNA, and washed at high stringency (65°C) in 0.2X SSC and 0.1% SDS. Both probes identified a band of approximately 3 kb. The 39mer gave a much stronger signal and was chosen for the primary screening of a cDNA library.

A human tonsil cDNA library (gift of L. Klickstein) was size-selected for inserts of 2kb or greater and constructed in λgt11 (Wong et al., 1985, Proc. Nat. Acad. Sci. U.S.A. 82:7711). The original library of 4 X 10⁶ recombinants was amplified once, and 200,000 recombinants plated at a density of 7500 plaques/100mm plate. The plaques were amplified in situ on duplicate nitrocellulose filters, as described by Woo (1979, Meth. Enzym. 68:389).

The oligonucleotide probes were labeled with ^{32}P -ATP using polynucleotide kinase. The filters were prehybridized for at least 2 hr at 42°C in 6 X SCC, 1 X Denhardts, 0.5% SDS, 0.05% phosphate buffer, and 100 $\mu\text{g}/\text{ml}$ of salmon sperm DNA. Hybridization with the 39mer was overnight at 42°C in prehybridization solution containing 20 $\mu\text{g}/\text{ml}$ tRNA. The filters were washed at 53°C to 55°C with 6 X SSC, 0.1% SDS, and 0.05% phosphate buffer. The damp filters were covered with plastic wrap and exposed to film with an intensifying screen. Phage that gave positive signals on duplicate filters were plaque purified and rescreened with the 39mer at a higher wash temperature (60°C) and with 20mer and 17mer mixed sequence probes. 15 positive clones were picked. Eight of the clones crossreacted with each other and gave positive signals with the 20mer mixed sequence probe and the independent 17mer mixed sequence probe. These clones were chosen for further analysis.

To confirm the identity of the cDNA clones, a 263 bp PstI/EcoRI restriction fragment which hybridized to the 39mer was subcloned into M13 vector and sequenced by the Sanger dideoxy chain termination method as follows. The amino acid sequence deduced from the DNA sequence is identical in 13 of 14 positions to the peptide sequence from which the 39mer probe was derived, including one amino acid which was not included in the design of the oligonucleotide. Furthermore, the predicted amino acid sequence shows that this peptide is preceded by a lysine and followed by an arginine, as expected for a tryptic fragment. The one mismatch may be due to normal polymorphism. The unique sequence oligonucleotide was 87% homologous to the cDNA sequence, despite the one amino acid mismatch.

The cDNA clones were restriction mapped by single and double restriction digests and, after end-labeling, by partial restriction digests (Maniatis et al., supra). Compatible

restriction fragments were subcloned directly into M13 cloning vectors. Other fragments were first blunt ended with Klenow, T4 polymerase, or Mung Bean nuclease (Maniatis et al., supra) and ligated into the HincII or SmaI site of the M13 polylinker. The nucleotide sequence of both strands was determined by the dideoxy chain termination method of Sanger et al. (1977, Proc. Nat. Acad. Sci. U.S.A. 74:5463) using ^{35}S -dATP.

The complete nucleotide sequence and deduced amino acid sequence of the β -subunit gene in the longest clone, 18.1.1 (2.8 kb is length), is shown in Figure 1. The first ATG is at position 73, and the sequence surrounding the ATG is consistent with the consensus rules for an initiation codon (Kozak 1984, Nucl. Acid. Res. 12:857). This putative initiation codon is followed by an open reading frame of 2304 bp, which could encode a polypeptide of 769 amino acids (aa). The stop codon ATC is followed by a 3' untranslated region of 394 bp. The poly A tail was not found, although a consensus polyadenylation signal (AATAAA) is located 9 bp from the 3' end.

The deduced amino acid sequence of the cDNA clones was compared to peptide sequence data from the beta subunit of Mac-1, LFA-1, and p150,95 (Fig. 2). In addition to the P61 and P-20 peptide sequences given above, one other peptide was sequenced from the beta subunit of p150,95. Tryptic peptides were also prepared and analyzed from the beta subunit of purified Mac-1 and LFA-1. Each peptide sequence is found within the deduced amino acid sequence (Figs. 1 and 2). Thus, it can be concluded that the cDNA encodes the β -subunit of human LFA-1.

The cDNA clones hybridize to a single mRNA species of approximately 3.0 kb, which is the same message identified by the 39mer oligonucleotide. This message is present in PMA-activated U937 cells (LFA-1 $^+$, Mac-1 $^+$, p150,95 $^+$), JY

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lymphoblastoid cells (LFA-1⁺, Mac-1⁻, p150,95⁻), and EBV-transformed cells from a normal donor (LFA-1⁺, Mac-1⁻, p150,95⁻), but is absent in HeLa cells (LFA-1⁻ Mac-1⁻, p150,95⁻). Although clone 18.1.1 lacks the poly A tail, it is close to the estimated size of the RNA message.

Within the deduced polypeptide are two regions of sufficient length and hydrophobicity that could span the membrane bilayer. The first domain, which begins with the putative initiation methionine and extends 22 amino acids, has the characteristics of a signal sequence. This putative signal sequence is followed by a charged glutamine, a residue which is often cyclized at the N-terminal position. This would be consistent with the N-terminal blockage of the β -subunit, if the signal sequence is cleaved during processing.

Use

The cDNA encoding the β -subunit of human LFA-1 can be used to produce recombinant β -subunit in large amounts. For example, the beta-subunit-encoding cDNA can be excised from the λ gt11 clones and introduced into an expression vector (plasmid, cosmid, phage or other type) to express the β -subunit in *E. coli*, using standard techniques. Alternatively the clones may be inserted into other vectors, such as mammalian, insect, or yeast expression vectors, and used to produce recombinant β -subunit in mammalian or yeast cells.

The subunits produced by the above methods can be readily purified and used as an immunogen to raise monoclonal antibodies to the subunits. These antibodies can be labelled and used in standard immunoassays to monitor the level of LFA-1, Mac-1, or p150,95 in white blood cells, and in the serum or other body fluids of patients having medical disorders associated with too many or too few cells having on their surfaces LFA-1 or related proteins. For example, diseases, e.g., AIDS, characterized by immunosuppression can be expected

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to be accompanied' by abnormally low levels of such cells, which are instrumental in fighting infections, and such diseases can thus be monitored by monitoring levels of these proteins. Also, other disease states, e.g., autoimmune disease, allograft rejection, and graft-versus-host disease, can be expected to be characterized by abnormally high levels of such cells, and thus also can be monitored by monitoring levels of these proteins. They can also be used to diagnose leukocyte adhesion deficiency, an inherited deficiency in the LFA-1, Mac-1, and p150,95 glycoproteins. Antibodies to the B-subunit can also be used to purify LFA-1 or related proteins by conventional immunoaffinity purification methods.

The purified proteins, particularly LFA-1, Mac-1 and/or p150,95, whether native or recombinant, can also be used therapeutically. The proteins can be administered to patients in need of such treatment in an effective amount (e.g., from 20-500 μ g per kg body weight), and mixed with a pharmaceutically acceptable carrier substance such as saline. Therapeutic utility of these proteins is based on the fact that disease states such as autoimmune diseases, allograft rejections, and graft-versus-host diseases involve abnormally high levels of cell-to-cell contact mediated by the recognition and binding of LFA-1 and related proteins to target antigen presenting cells, endothelial cells, and other types of cells. The administration of LFA-1 or a related protein, or fragments thereof, will compete for receptors for the cell-bound protein, inhibiting cell-to-cell binding and thus bringing about the desired immunosuppression. A particular disease for which these proteins will be useful is the autoimmune disease rheumatoid arthritis. Preferably administration is intravenous at about 20-500 μ g per kg body weight, or directly at an inflamed joint of a patient suffering from rheumatoid arthritis. Alternatively, oral administration or local application can be used by providing

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tablets, capsules, or solutions, or by applying lotions as required. The amount and method of administration will vary dependent upon the age and weight of the patient, and the disease to be treated. Other autoimmune diseases which can be treated include systemic lupus erythematosis, juvenile onset diabetes, multiple sclerosis, allergic conditions, eczema, ulcerative colitis, inflammatory bowel disease, Crohn's disease, as well as allograft rejections (e.g., rejection of a transplanted kidney or heart). LFA-1, Mac-1, and p150,95 normally act in situ by binding to endothelial and other cells. Thus, the free proteins or peptides, which are administered, will be able to inhibit leukocyte immune responses and migration to inflammatory sites.

The β subunit cDNA clone can be used in prenatal diagnosis of leukocyte adhesion deficiency (LAD). LAD disease is a deficiency in cell surface expression of LFA-1, Mac-1, and p150,95 and is due at least in part to a primary genetic lesion in the β subunit. Patients with the severe form of LAD disease suffer from recurrent bacterial infections and rarely survive beyond childhood. The defect can be detected early in pregnancy since it is associated with a unique restriction fragment length polymorphism. PstI digestion of human DNA and hybridization with the 1.8 kb EcoRI fragment (shown in Fig. 2) of the β subunit cDNA defines a restriction fragment length polymorphism (RFLP). Diagnosis of this disease is therefore performed by standard procedure using the whole or a part of this EcoRI fragment. The genomic DNAs of the parents of the fetus, and the fetus are screened with this probe and an analysis of their RFLPs made. In this way the probability that the fetus has the disease can be estimated.

ICAMs (such as ICAM-1) are recognized by certain human viruses (particularly rhinoviruses of the major type (which bind to ICAM-1). These viruses bind to human cells by virtue of this recognition, and thereby mediate viral infection.

Thus, a central step in the etiology of viral disease is the interaction between these cellular receptors and the virus.

Agents which suppress, compete with, or inhibit the ability of a virus to bind to an ICAM molecule thus have use in the treatment of viral (and particularly rhinoviral) infection.

One aspect of the present invention thus concerns the ability of the beta-subunit of human LFA-1, and its functional derivatives to interact with ICAM-1 and to thereby either prevent cell-viral attachment and viral infection, or to attenuate or diminish the severity or duration of such infection.

Of particular interest to the present invention are functional derivatives of the β -subunit of human LFA-1 such as solubilized forms of the β -subunit of human LFA-1, fragments of the β -subunit of human LFA-1, etc. Such agents are preferably provided to a recipient patient as a heterodimer containing the molecule in association with a molecule of the α -subunit of a member of the CD-18 family (such as the α -subunits of LFA-1, p150,95, or Mac-1). The above-described goal of treating viral infection may be accomplished with a single agent or with a combination of more than one agents.

For the purpose of treating viral infection, the above-described agent(s) of the present invention is to be provided to a recipient patient (for example, by intranasal means) at a dosage sufficient to permit the agent(s) to suppress, compete with, or inhibit the ability of a virus to bind to an ICAM molecule. Such a dosage shall, in general, be (for each agent provided) from 0.01 pg/kg patient weight to 1 mg/kg patient weight, although greater or lesser amounts can be employed.

For the purpose of treating viral infection, the administration of such agent(s) may be provided either "prophylactically" or "therapeutically." When provided

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prophylactically, the agent(s) are provided in advance of (i.e. prior to, at, or shortly after) the time of infection but in advance of any symptoms of viral infection. The prophylactic administration of the agent(s) serves to prevent or attenuate any subsequent infection. When provided therapeutically, the agent(s) are provided at (or shortly after) the onset of a symptom of actual viral infection (such as, for example, the appearance of virally induced nasal congestion, etc. or the detection of virus in bodily fluids, or the detection of antibodies, directed against the virus, in the serum of an infected patient, etc.). The therapeutic administration of the agent(s) serves to attenuate any actual infection, and thus lessen its severity or duration.

Other embodiments are within the following claims.

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Claims

1. A method of treating viral infection which comprises providing to a subject in need of such treatment, an effective amount of a composition comprising the β -subunit of human LFA-1, or a functional derivative thereof.
2. The method of claim 1 wherein said β -subunit of human LFA-1 is provided in association with an alpha chain of a member of the CD-18 family.
3. The method of claim 1 wherein said viral infection is a rhinoviral infection.
4. The method of claim 2 wherein said viral infection is a rhinoviral infection.

SUBSTITUTE SHEET

880	GGC GGC GAC GAG CTC GGC GGC AAC CTC ACC TCG AAC GAC GGC CTC GTC GTC AAC TAC CCA TCG GTC GGC Ala Gly Asp Gly Lys Leu Glu Ala Ile Leu Thr Pro Asn Asp Gly Arg(Cys)His Leu Glu Asp Asn Leu Tyr Lys Arg Ser Asn Glu Phe Asp Tyr Pro Ser Val Gly	305
988	CAG CTG GCG CTC AAG CTC CCT GAC AAC TTC TCG GTC AAC AGT ATG ACC TAC GAG AAC CTC ACC AGT CCC AUG TCA GCG Gln Leu Ala His Lys Leu Ala Glu Asn Asn Thr Ser Glu Pro Ile Phe Glu Asn Asn Thr Ser Arg Het Val Lys Thr Tyr Glu Lys Leu Thr Glu Ile Pro Lys Ser Ala	341
1096	GTC GGG GAG CTG TCT GAG GAC TCC AGC ATT ATG GTC GTC CAT CTC AAC GGC CTC CCC GTC ACC Val Glu Glu Leu Ser Glu Asp Ser Ser Asn Val Val His Leu Ile Lys Asn Ala Tyr Asn Ala Leu Pro His Asn Ala Leu Pro Asp Thr H-52 L-65	377
1204	CTG AA GTC ACC TAC GAC TCC TTG TGC AGC ATT GTC GTC AAC GAG CTC ATT GAC TGT GAT GGC GTC GAG ATC AAC TTC CAG GTC Leu Lys Val Thr Tyr Asp Ser Phe(Cys)Ser Asn Glu Val Thr His Arg Asn Glu Pro Arg Glu Asp(Gly)Asp Glu(Gly)Val Glu(Gly)Val Val Pro Glu(Gly)Asp(Gly)Val Val Val	413
1312	AAC GTC AGC ACA GAG TCC ATC CTC GCG CTC GCG ATT GTC AAC GTC AAC GAG CTC ATT GTC AAC GTC ATT GGG AM AAC TGT GAG TCC CAG ACA Lys Val Thr Ala Thr Glu(Cys)Ile Glu Glu Glu Ser Phe Val Ile Arg Ala Leu Glu(Cys)Gly Ile Val Thr Asp Ile Val Thr Val Glu(Gly)Arg(Cys)Asp Thr Val	449
1420	GG GAC GAG AGC AGC ACC CTC TCC CTC GTC GGC TCC AAC GTC AAC TAC GAC ACT GGC TAC ATT GGG AM AAC TGT GAG TCC CAG ACA Arg Asp Glu Ser Arg Asp Arg Ser Leu(Cys)His Glu(Cys)Gly Ile Leu Glu(Cys)Gly Ile Val Thr Ile Glu(Cys)Asp Asn Asn Ser Ile Ile(Cys)Ser Glu(Gly)Leu(Gly)Asp(Gly)Leu(Gly)Asp(Gly)Ser(Gly)Val(Cys)Val(Cys)Gly Glu(Cys)Ile(Gly)His Thr	485
1528	CAG GCG AGC AGC GAG CTC GTC GCA GAA ACC TCC CGG CTC GGC TCC GAG TCC GTC GAG TCC CTC GTC GAC ACC Gln Gly Arg Ser Ser Ser Glu Glu Leu Glu Glu Glu Ser(Cys)Arg Lys Asp Asn Asn Ser Ile Ile(Cys)Ser Glu(Gly)Leu(Gly)Asp(Gly)Leu(Gly)Asp(Gly)Ser(Gly)Val(Cys)Val(Cys)Gly Glu(Cys)Ile(Gly)His Thr	521

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FIG. 1 (CONTINUED PAGE 2)

FIG. 1 (CONTINUED PAGE 3)

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p150.95 β Subunit

P-61 sequence	L Y E N N I Q P I F A V T S
Deduced sequence	K L A E N N I Q P I F A V T S
P-20 sequence	(T/C) D T G Y I G K
Deduced sequence	R C D T G Y I G K
P-18 sequence	S S Q E L E G S (T/C) (R)
Deduced sequence	R S S Q E L E G S C R

Mac-1 β Subunit

M-58 sequence	L L V F A T D D G F H F
Deduced sequence	R L L V F A T D D G F H F
M-52 sequence	X A V G E L S E X (S) X N
Deduced sequence	K S A V G E L S E D S S N

LFA-1 β Subunit

L56a sequence	E C Q P P F A F R
Deduced sequence	K E C Q P P F A F R
L56b sequence	L I Y G Q Y C E (C) D T I
Deduced sequence	K L I Y G Q Y C E C D T I
L-65 sequence	V F L D H N A L P
Deduced sequence	R V F L D H N A L P

FIG. 2

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/01255

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07K 15/14; 13/00

U.S. CL.: 514/8,12; 530/395

II. FIELDS SEARCHED

Classification System	Minimum Documentation Searched ?	
	Classification Symbols	
U.S. CL.	514/8,12	530/395

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched *

Protein Sequence Data bases, DIALOG and APS Data bases for sequences, LFA, Mac-1, p150,95,CD18 and viral, virus? and rhinovir?

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y,P	Cell, published 10 March 1989, "A Cell Adhesion Molecule, ICAM-1, is the Major Surface Receptor for Rhinoviruses", (STAUNTON), Vol. 56, pages 849-853. See pages 849, 850 and 852.	1-4
Y	European Journal of Immunology, Published August 1988, "The Role of Lymphocyte Function-associated Antigen (LFA-1) in the Adherence of T Lymphocytes to B Lymphocytes", (Mazerolles), Vol. 18, pages 1229-1234. See pages 1229, 1231, and 1232.	1,2
A	The Journal of Immunology, Published 15 August 1986, "A Human Intercellular Adhesion Molecule (ICAM-1) Distinct from LFA-1", (Rothlein), Vol. 137, pages 1270-1274. See page 1270.	1-4
A	European Journal of Immunology, Published 1987, "LFA-1 beta-chain Synthesis and Degradation in Patients with Leukocyte-adhesive Proteins Deficiency", (Dimanche), Vol. 17, pages 417-419. See pages 417 and 419.	1-4

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

05 MAY 1990

Date of Mailing of this International Search Report

11 JUN 1990

International Searching Authority

ISA/US

Signature of Authorized Officer
Veronica Erne for
KEITH C. FURMAN